Synchrotron radiation is helping to identify tiny amounts of paint, drugs and fibres found at crime scenes

Physics and forensics

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FORENSIC science is rarely out of the news these days. Hardly a week seems to pass without reports of a criminal being convicted by DNA evidence extracted from a single hair, a flake of skin or a trace of blood or saliva found at a crime scene. Less well known, however, is the way in which forensic science has benefited from our ability to identify the presence of molecules in a sample using infrared radiation.

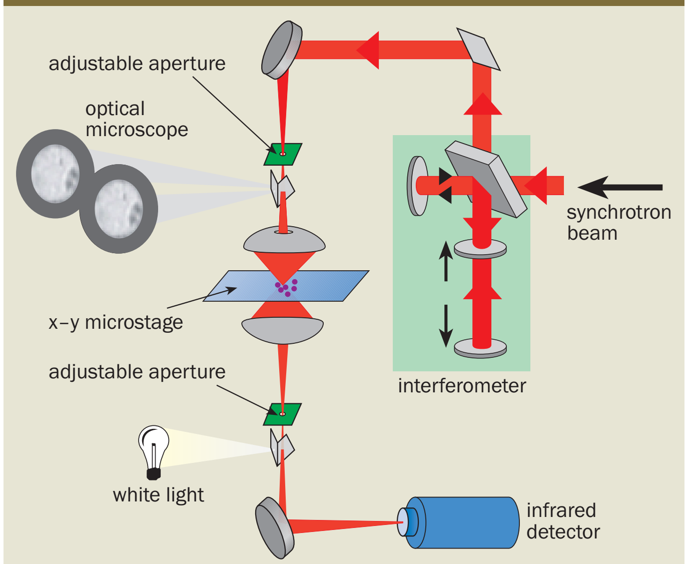
Unfortunately, many of the materials encountered at crime scenes are too small to be analysed using standard instrumentation, even at infrared wavelengths. However, the advent of "infrared spectromicroscopy" has led to significant advances in forensics.

From an analytical standpoint, infrared spectroscopy is extremely powerful because infrared photons can couple directly to certain vibrational modes of molecules. Targeting these vibrational modes provides a unique signature for many molecules. Ultraviolet light, on the other hand, excites broad electronic transitions, which means that it cannot, in general, uniquely identify samples.

Measuring the frequency of vibrational modes using infrared spectroscopy reveals the molecular fingerprint of the compounds in a sample. Such fingerprints are invaluable for identifying particular molecules, their structure and the chemical reactions they undergo. Indeed, infrared spectroscopy has already been used to study a diverse range of samples, including crops, soil, thin films, powders and a wealth of biological materials.

It is therefore hardly surprising that infrared spectroscopy has begun to play a significant role in forensics. Indeed, the technique has become one of the work horses of the standard forensics laboratory, examining everything from drugs, paints, fibres and explosives to polymers, inks and documents. And it has proved a highly effective tool for analysing samples from crime scenes, such as blood, fabrics and soil particles. Crucially, infrared spectroscopy allows crime investigators to compare a sample with a known compound or item in order to determine whether they share any chemical or physical properties.

1 A close-up of an infrared spectromicroscope



Infrared light from a thermal source or a synchrotron beam enters the Michelson interferometer from the right. The modulated light leaves the interferometer and enters an infrared microscope, where it is focused onto a sample. The sample absorbs certain frequencies of light and the outgoing components of light are focused onto an infrared detector. Visible images of the sample can also be taken at the same time. The sample is positioned on a computer-controlled microstage so that the infrared spectrum can be mapped with micron precision.

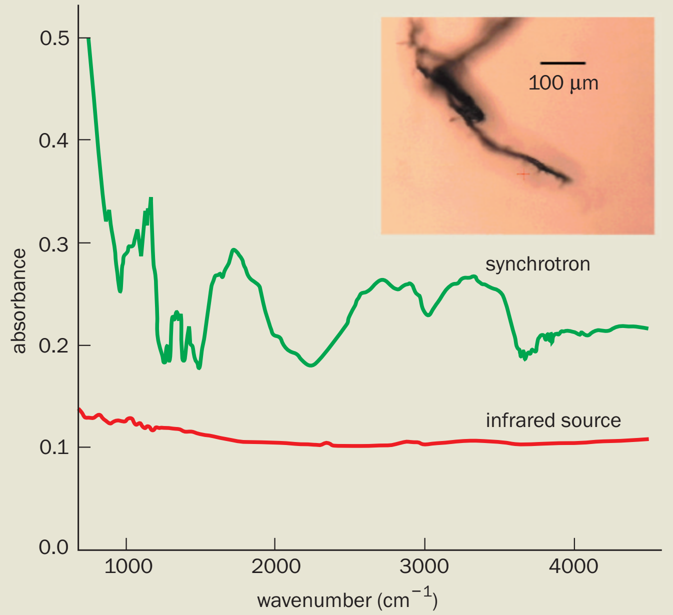
Infrared radiation to the rescue

Powerful as it is, the use of infrared spectroscopy in forensics applications has been limited by our inability to examine relatively small amounts of material with high spatial resolution and spectral sensitivity. The first team to combine microscopy and infrared spectroscopy was led by R Barer of Oxford University in the UK in 1949. Simply speaking, Barer and co-workers passed a beam of infrared radiation through a diffraction grating to separate it into different wavelengths and then focused part of the outgoing light through a microscope onto the sample. Using a second microscope to detect the reflected light, they painstakingly measured the absorption as a function of frequency. These ideas led to the development of the first commercial microscope for infrared spectroscopy in 1953.

In the past decade, however, spectromicroscopy has been revolutionized thanks to high-quality optics, inexpensive spectrometers and fast computers to process the data. Modern infrared spectromicroscopes typically use the thermal emission from a filament heated to between 1000 and 2000 K. This light is then collected by a series of reflective optical elements and passed to a Michelson interferometer, rather than a diffraction grating, to separate the light into different wavelengths (see figure 1, courtesy of P. Dumas). Michelson interferometers are used because they can transmit far more light than gratings. Moreover, they have a high intrinsic resolution, they can be calibrated easily, and allow measurements to be made quickly.

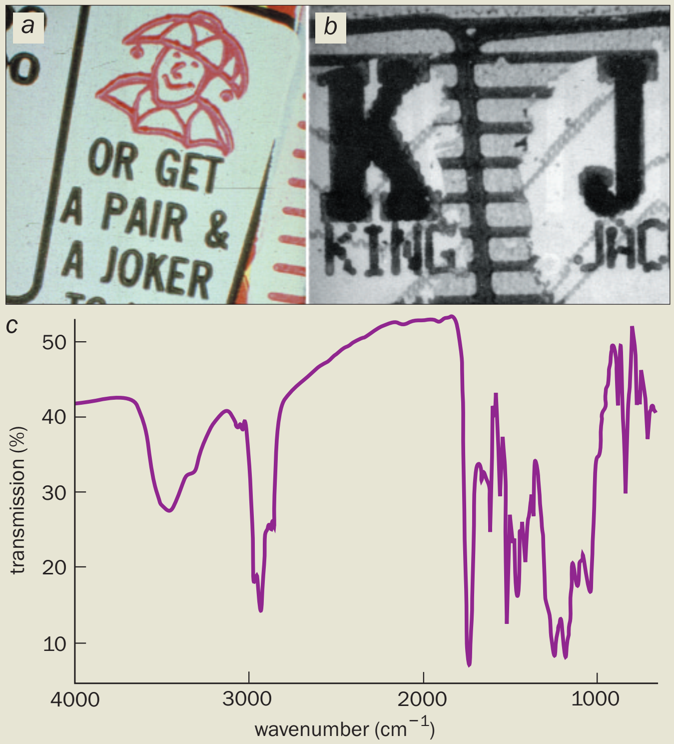
The interferometer splits the incoming light into two beams

2 Synchrotrons reveal the details



The absorbance of an ink-soaked paper fibre (inset) as a function of wavenumber, the number of waves per centimetre. The green spectrum was obtained using infrared microscopy at the Advanced Light Source, a synchrotron source that is some 200 times brighter than a conventional black body. In contrast, the red spectrum was measured under the same experimental conditions and over the same period of time but with a conventional thermal source. The improvement in brightness at the synchrotron enhances the signal-to-noise ratio for small samples.

3 Infrared spectra hit the jackpot



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(*a*) Part of the suspicious lottery scratch card. (*b*) The portion of the lottery ticket that is scratched off to reveal the prize. (*c*) The spectrum obtained using infrared microscopy.

– one of which reflects from a fixed mirror while the other reflects from a movable mirror – and then recombines them. This modulated light is then passed through an infrared microscope that focuses the light onto a small spot on the sample. Next the intensity of the light reflected by the sample is measured by an infrared detector as a function of the mirror position. Sometimes the light transmitted by the sample is used instead, in particular if the sample is very thin. The resulting interferogram is then Fourier transformed to reveal the different frequency components of the signal. This spectrum is simply the spectrum of the incoming light modulated by the frequencies absorbed or emitted by the sample.

With a thermal emitter, samples some 75 microns in size can now be routinely visualized and characterized in forensics laboratories. Recently, scientists have further decreased the size of the samples that can be studied by using synchrotron radiation in conjunction with infrared spectromicroscopy. Intense synchrotron beams allow the tiny amounts of materials that are collected at crime scenes to be studied.

Synchrotrons produce a broad spectrum of radiation, from the far infrared to X-rays. Even though every synchrotron beam produces infrared radiation, only a few existing facilities in the US, France, the UK and, most recently, Germany, have been configured to extract and use this bright source of infrared light.

So far, the results have been impressive. Spectra at mid-infrared wavelengths – 3 to 20 microns – have been obtained from specimens measuring less than 10 microns across and weighing a few femtogrammes (10^{-15} kg). Such a resolution is "diffraction limited" and is very close to what could be achieved with an ideal point source. Indeed, forensic scientists are now able to study samples at a size and concentration level that has until now been impossible.

Synchrotron radiation is hundreds of times brighter than conventional infrared sources because it can be focused to spot sizes just a few microns across. This increased brightness directly translates into a higher signal-to-noise ratio for small samples (see figure 2). In spite of the high brightness, synchrotron infrared light does not degrade the sample because it does not break any bonds or change the chemical formula. Rather, it is absorbed by the vibrational and rotational modes of molecules with an electric dipole moment. Indeed, studies have shown that focused synchrotron infrared light increases the temperature of biological samples by a mere 0.5 K.

The success of synchrotron infrared spectromicroscopy has motivated every new synchrotron facility that is being planned or is under construction to include at least one beam line dedicated to such experiments.

Crime watch

So how has infrared spectroscopy helped in the fight against crime? Back in 1989, the authenticity of an instant-win lottery scratch card was questioned when it was submitted as a winning ticket in Connecticut in the US. Computer records showed that the ticket was not a winner based on its pre-printed serial number. Attempts to forge or alter lottery tickets are fairly common, so forensic staff at the lottery agency analysed the scratch card in detail. They could find no alteration or forgery. Since a great deal of prize money was at stake, the ticket was sent to a private company called Spectra-Tech for further analysis (see figure 3). Scientists there obtained infrared spectra from various parts of the ticket – in particular around the scratch panels and the serial number – and compared them with spectra from valid tickets. When the two sets of data were shown to match, the problem was deemed to be a "computer error" and the winnings were paid out.

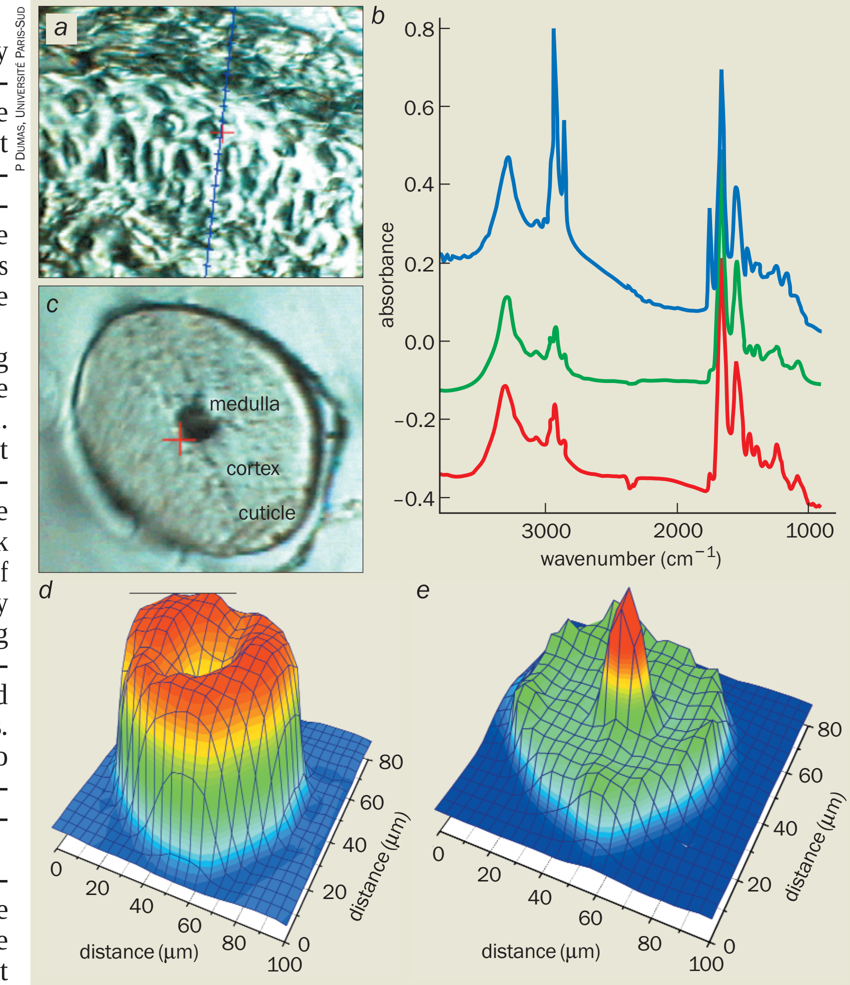
Like the lottery card, forensic scientists often try to identify other suspect documents from the infrared spectra of the ink, toners or markers, or the paper itself. The spectrum of ink on counterfeit banknotes, for example, does not match the spectrum obtained from real currency. Forensics experts often use such data to identify the possible origin of the paper, to verify that the document is as old as it is claimed to be, and to check that the same ink is used throughout the document.

However, one of the difficulties of analysing ink is that the pigment must be extracted before it can be examined with infrared radiation. Typically, a hole is punched in the document with a hypodermic needle and the ink is separated chemically from the paper. Such destructive procedures require complex laboratory work and, more seriously, may alter the chemistry of the ink being analysed. Our group at Berkeley has overcome these problems, however, using synchrotron radiation at mid infrared wavelengths (2.5–25 microns) from the Advanced Light Source (ALS) to characterize pigments. The radiation is so intense that it allows us to make rapid and direct spectroscopic measurements of inks without having to chemically separate them from the paper.

Infrared spectroscopy has also proved a powerful tool in the fight against illegal drugs. Here the challenge for forensic chemists is to identify the compounds in the sample unambiguously so that the evidence stands up in a court of law. The best results are achieved with pure samples but in many cases other techniques are needed to first purify or isolate the sample. This is often the case for illegal drugs that have been sold on the street and that are usually mixed with other substances.

In one case in the late 1990s, police in Ottawa, Canada, identified a derivative of an amphetamine from a small piece of evidence using a combination of infrared spectroscopy and other techniques. Moreover, they discovered small amounts of another contaminant in the sample that suggested that the drug had been manufactured illegally.

4 Skin and hair under the microscope



(a) An optical image of a cross section of human skin reveals three layers. (b) Spectra from each layer of skin – the *stratum corneum* (blue), the *stratum granulosum* (green) and the *dermis* (red) – as a function of wavenumber show that each layer contains similar amounts of the protein amide (1500–1700 cm^{-1}). Meanwhile, the peaks between 2850–3000 cm^{-1} and around 1750 cm^{-1} indicate a greater abundance of carbon–hydrogen bonds and carboxyl groups in the *stratum corneum* than in the other layers. (c) An optical image of a cross section of a human hair shows the medulla, cortex and cuticle, each made of different kinds of dead cells. (d) The absorption of the protein amide as a function of position, as measured by infrared spectromicroscopy. The results demonstrate that the protein is concentrated in the cortex. (e) The absorption spectra of a carbon–hydrogen bond in a fatty lipid molecule as a function of position. This graph shows that lipids are concentrated in the medulla, the core of the hair fibre.

In most cases, infrared spectroscopy is combined with other analytical techniques. Chips of paint, for example, are first examined with an optical microscope to determine the colour, layering and texture. This initial screening can often reveal whether the paint comes from a house, a car or a boat. Infrared spectroscopy, however, helps forensic analysts to determine the type of paint and its main components. In hit-and-run cases, for example, experts first compare paint chips found on the body or at the crime scene with a database of known samples and then use infrared spectroscopy to identify the year, make and model of the car – crucial investigative leads for the police. Paint chips offer compelling proof when they consist of multiple layers, as each layer can be analysed to give a better match to a crime scene.

Infrared spectroscopy also plays an integral part in the analysis of various types of fibres, and in many cases it plays a crucial role in identifying the composition of the fibre unequivocally. When police in Kern County, California, were investigating a murder, they found small fragments of red polyester fibre on the victim's body. Using infrared spectromicroscopy, analysts from the forensic-science division of the district attorney's office matched these fibres to those found on the seats of the prime suspect's car. The police also found a fragment of a plant that only grows in the salt marshes where the victim was dumped. The suspect was convicted on the basis of this evidence.

Bright outlook for synchrotrons

The future applications of synchrotron-based infrared spectromicroscopy to forensics are virtually limitless. In addition to simple material and chemical samples, the technique lends itself nicely to the study of more complex systems. Crime scenes offer many examples of composite vibrationally active samples, including blood smears on surfaces, mixed tissue and body fluids. Infrared spectromicroscopy is ideal for examining the minute traces of blood found at crime scenes because high spatial resolution and high sensitivity are absolute musts.

Yolanda Duvalt and co-workers at the cosmetics company L'Oréal, in Paris, have already demonstrated that complex biological samples can be analysed effectively with synchrotron infrared spectromicroscopy. They are using the technique to study the microscopic biochemical changes that occur in human hair when various haircare products are applied (see figure 4 courtesy of P. Dumas and *Physics World* May 2001 pp22–23). Indeed, the various components of hair, nails and skin have different vibrational spectra, making it possible to fingerprint them and compare them with other similar samples. Infrared examination of these types of tissue may allow investigators to resolve issues such as the degree of decomposition of a body and the cause of death.

Another area that has remained relatively unexplored, at least from a forensic standpoint, is the study of trace amounts of fluids or chemicals on surfaces. Obvious examples include biological fluids on clothing, blood on glass and fingerprints on a weapon. Any object found at a crime scene is likely to have chemical molecules with vibrational modes on the surface. Knives, guns and other weapons are likely to be covered in gunpowder residue, dust or cleaning oil, rather than being completely clean. All these contaminants will have their own infrared spectra that can provide a total chemical picture of the weapon. Gunpowder from different manufacturers and suppliers, for example, may have different chemical formulations and thus a unique vibrational signature. Even dust particles should have unique spectra because of their different chemical and geological origins. Due to the increased brightness of the synchrotron infrared source, such tiny traces could be examined using infrared spectromicroscopy.

Indeed, at the microscopic level, the use of synchrotron-based infrared fingerprinting looks bright. Studies of trace amounts of explosives, tiny fragments of paint and plastic from cars, clothing, carpet fibres and hair dyes are all possible. Illicit drugs and poisons can be examined too. Post-mortem changes in hair have been detailed in the forensics literature and, in light of the extreme detail already reported using infrared spectroscopy, infrared radiation is likely to play an even greater role in the future of forensics.

Further reading

R L Brunelle and R Reed 1984 *Forensic Examination of Ink and Paper* (Charles C Thomas Pub Ltd, Springfield)

R Saferstein (ed) 2001 *Forensic Science Handbook (Volume 1)* (Prentice Hall, New Jersey) 2nd edn

John Vassallo (ed) 1995 Special issue on synchrotron infrared spectroscopy *Synchrotron Radiation News* **8** (5)

Links

infrared.als.lbl.gov

infrared.nsls.bnl.gov

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